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14. ABSTRACT
The overall objectives of our work on human ovarian carcinoma cells are to determine the mechanisms whereby hyaluronan-CD44-CD147 interactions influence malignant cell behavior and therapy resistance, and to apply our findings to the improvement of therapy, in malignant ovarian carcinoma. In this grant period we have shown that small hyaluronan oligosaccharides, which have previously been shown to antagonize hyaluronan-receptor interactions, sensitize cisplatin-resistant human ovarian carcinoma cells to cisplatin treatment in a mouse xenograft model, as well as in cell culture. Hyaluronan oligosaccharide-decorated nanoparticles containing chemotherapeutic agents are in preparation for maximizing these effects. We have also shown that siRNAs against the hyaluronan receptor, CD44, and the regulator of hyaluronan synthesis, CD147, sensitize cisplatin-resistant ovarian carcinoma cells to cisplatin in cell culture. Nanoparticles containing these siRNAs are in preparation for use in vivo.

15. SUBJECT TERMS
Ovarian carcinoma; hyaluronan; CD147; CD44; drug resistance

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INTRODUCTION

Our previous studies have shown that hyaluronan, the hyaluronan receptors CD44 or LYVE-1, and the Ig superfamily member CD147 act cooperatively to promote malignant and drug-resistant properties. This most likely occurs through assembly and/or stabilization of plasma membrane signaling complexes containing CD44 or LYVE-1 in association with CD147, receptor tyrosine kinases and transporters implicated in malignancy and resistance to therapies (Ghatak et al., 2005; Grass et al., 2012; Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c). CD147 (emmprin; basigin) is a cell surface member of the Ig superfamily that induces expression of hyaluronan and matrix metalloproteinases, and promotes cell invasiveness, anchorage independent growth, drug resistance, and tumor growth and metastasis *in vivo* (Caudroy et al., 2002; Dai et al., 2013; Marieb et al., 2004; Zucker et al., 2001). We have shown recently that sub-populations of ovarian carcinoma cells, and other cancer cell types, with high expression of cell surface CD147 have similar properties to cancer stem cells, including enhanced levels of anchorage-independent growth, drug resistance and invasiveness (Dai et al., 2013).

Many of the activities of CD147 in cancer cells are dependent on hyaluronan-CD44 or LYVE-1 signaling (Ghatak et al., 2005; Marieb et al., 2004; Misra et al., 2003; Qin et al., 2011) and CD44 is one of the most common markers for carcinoma cancer stem cells (Zoller, 2011). The overall objectives of our work are to determine the mechanisms whereby hyaluronan-CD44-CD147 interactions influence malignant cell behavior and therapy resistance, and to apply our findings to the improvement of therapy, in particular in recurrent ovarian carcinoma. For example, we have found that multivalent interactions of hyaluronan polymer with CD44 are necessary for stabilizing CD44-CD147 signaling complexes, and that small, monovalent, hyaluronan oligosaccharides antagonize hyaluronan-receptor signaling by disrupting constitutive hyaluronan polymer-receptor interaction, thus leading to inhibition of oncogenic signaling pathways, chemoresistance and malignant characteristics (Ghatak et al., 2002; Ghatak et al., 2005; Gilg et al., 2008; Misra et al., 2006; Qin et al., 2011; Slomiany et al., 2009a; Slomiany et al., 2009b; Slomiany et al., 2009c). In particular we have found that treatment with small hyaluronan oligosaccharides is effective in sensitizing various types of drug-resistant cancer cells to chemotherapeutic agents (Gilg et al., 2008; Misra et al., 2005; Misra et al., 2003; Qin et al., 2011; Slomiany et al., 2009a). Most notably, these oligosaccharides inhibit tumor growth by drug-resistant cancer stem cell sub-populations obtained from human patient-derived ovarian carcinoma cells (Slomiany et al., 2009b).

Our aims for this grant are to establish efficacy for small hyaluronan oligosaccharides as chemo-sensitizing agents in xenograft models of human ovarian carcinoma cells, to determine whether siRNAs directed against CD44 and CD147 affect ovarian carcinoma chemoresistance and metastasis, and to explore mechanisms for increasing efficiency of delivery of these agents together with chemotherapeutic agents.

BODY

In this grant period, our major aim has been to assess various parameters of treatment with small hyaluronan oligosaccharides and siRNAs against CD44 and CD147 using the cisplatin-resistant human ovarian carcinoma cell line, A2780cp20. We chose this cell line since cisplatin is commonly used in first-line treatment of ovarian carcinoma patients. The results of these experiments will be used to guide our experiments with ovarian carcinoma cancer stem-like cells obtained from patient ascites fluids.

a) **Chemo-sensitization by small hyaluronan oligosaccharides *in vitro***

In our preliminary experiments we showed that co-treatment with small hyaluronan oligosaccharides sensitized human ovarian carcinoma cells (SKOV-3 and PE04) to various chemotherapeutic agents, i.e. taxol, doxorubicin and cisplatin, in cell culture (Figure 1).

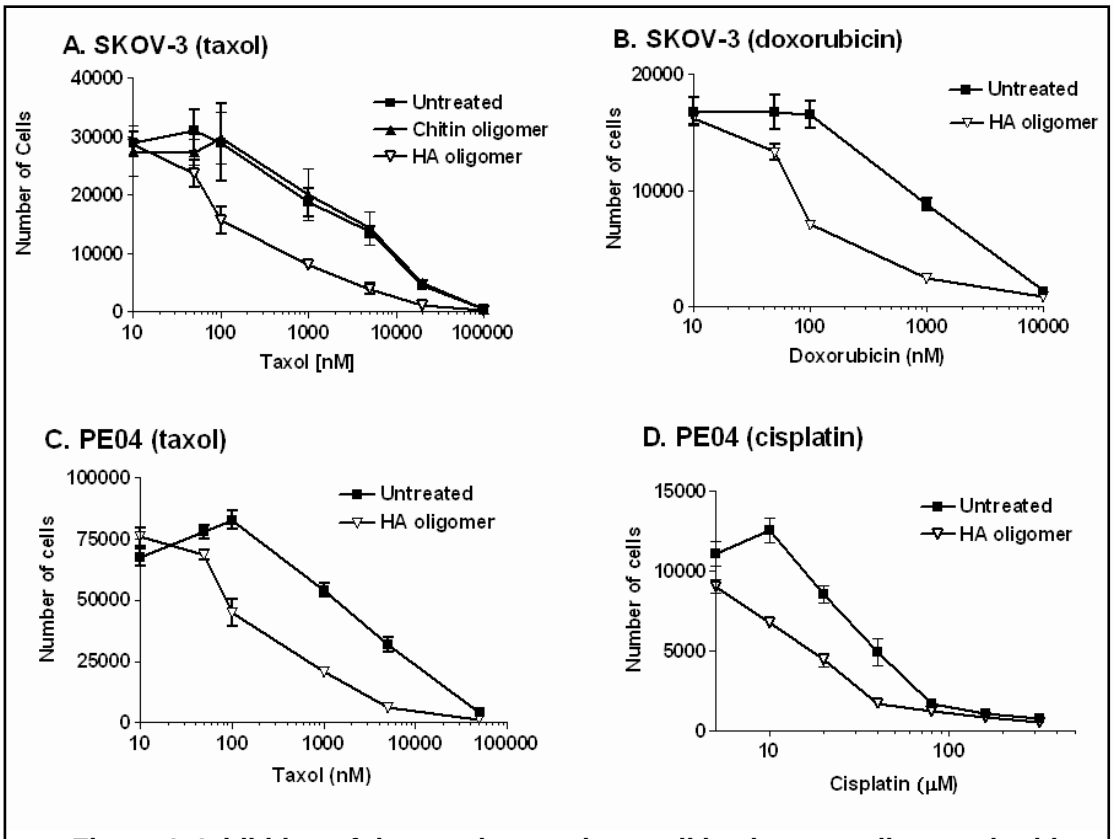

Figure 1: Inhibition of drug resistance by small hyaluronan oligosaccharides (HA oligomer). A-D: SKOV-3 and PEO4 human ovarian carcinoma cells were treated for 24 h with a range of concentrations of paclitaxel, doxorubicin, or cisplatin in the presence or absence of 100 μg/ml oHA, and then assayed for cell survival. In A, chitin oligomers were used as a negative control.

b) **Chemo-sensitization by small hyaluronan oligosaccharides *in vivo***

Using intraperitoneal xenografts of cisplatin-resistant A2780cp20 human ovarian carcinoma cells, we have now shown that co-treatment with small hyaluronan oligosaccharides sensitizes these cells to treatment with cisplatin *in vivo* (Figure 2). The oligosaccharides also sensitized these cells to treatment with doxorubicin *in vivo* (Figure 2). Doses of cisplatin and doxorubicin were chosen to give sub-optimal responses when used alone. Co-treatment with the hyaluronan oligosaccharides plus either drug caused large decreases in tumor growth, as compared to drug treatment alone (Figure 2).

In collaboration with Dr. Anil Sood's group at MD Anderson, we have also prepared small hyaluronan oligosaccharide-decorated chitosan nanoparticles (Han et al., 2011), which will be used to determine whether these particles, with and without loading with cisplatin, doxorubicin or taxol, are more efficient than the same reagents in solution.

In addition, we have begun to prepare ovarian carcinoma cells from patient-derived ascites fluids by previously developed methods (Slomiany et al., 2009b), in order to test the effects of small hyaluronan oligosaccharides on cisplatin and taxol resistance in these cells.

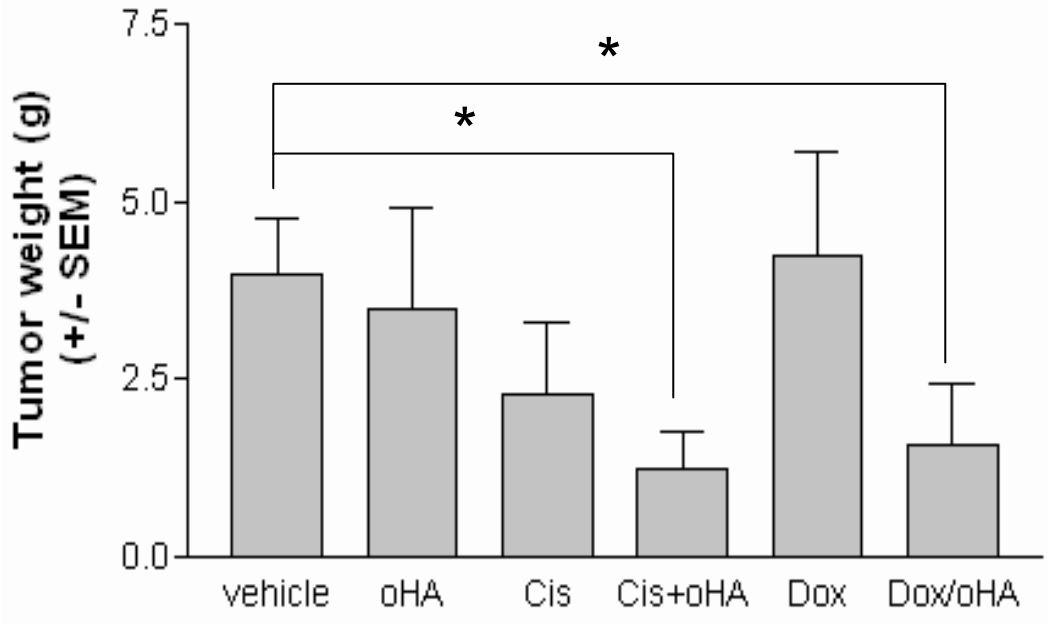

Figure 2: Chemo-sensitization *in vivo* of cisplatin-resistant ovarian carcinoma cells with small hyaluronan oligosaccharides (oHA). A2780cp20 human ovarian carcinoma cells ($1x10^6$ per mouse) were injected intraperitoneally. Groups of 5 mice were treated with vehicle (200ul saline) or the following reagents dissolved in 200ul saline: 10mg/kg oHA, 5mg/kg cisplatin (cis), 5mg/kg cisplatin + 10mg/kg oHA, 0.05mg/kg doxorubicin (dox), or 0.05mg/kg doxorubicin + 10mg/kg oHA. Treatments were administered intraperitoneally once per week for 4 weeks. The mice were then sacrificed and peritoneal tumors weighed. *p value <0.05

c) **Chemo-sensitization by siRNAs against CD44 and CD147**

Despite previous success in obtaining efficient knockdown in various cancer cell types with siRNAs against CD44 and CD147 (Ghatak et al., 2005; Grass et al., 2012; Misra et al., 2005; Qin et al., 2011; Slomiany et al., 2009c), we have had difficulties obtaining efficient knockdown in A2780cp20 cisplatin-resistant ovarian carcinoma cells. In recent attempts, we have succeeded in obtaining partial but significant knockdown (Figure 3). Using these conditions we have also shown partial effects of these siRNAs on cisplatin resistance in the A2780cp20 cells in culture (Figure 4).

In order to obtain more robust effects we are collaborating with Anil Sood's group at MD Anderson to encapsulate the siRNAs in liposomal nanoparticles. Similar particles have been used with considerable success in their studies of other anti-ovarian carcinoma siRNAs *in vitro* and *in vivo* (Landen et al., 2010; Mangala et al., 2009; Spannuth (Graybill) et al., 2011).

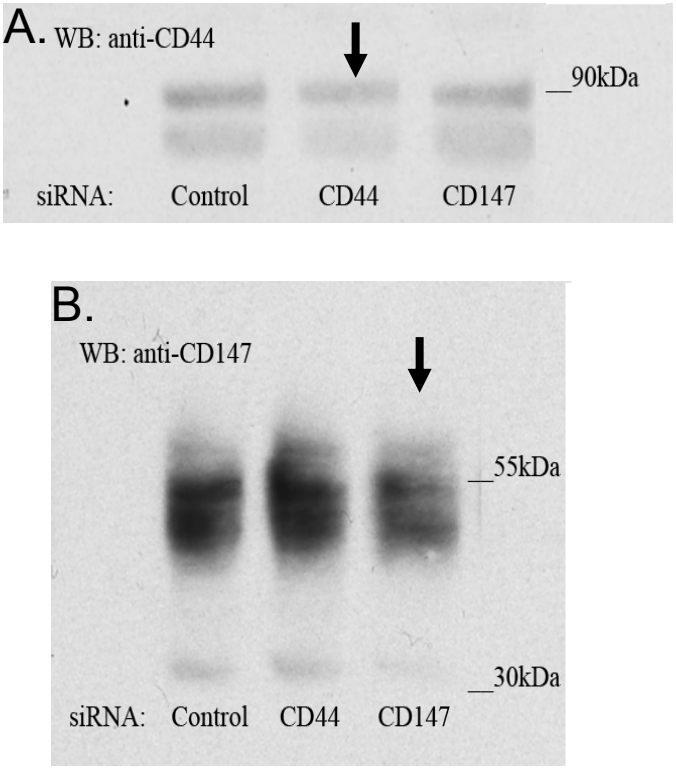

Figure 3. Partial knockdown with siRNAs for CD44 and CD147. A2780cp20 cells were treated with pooled siRNAs for CD44 or CD147, or with control siRNA, and then processed for Western blotting with antibodies against: **A.** CD44 or **B**. CD147. Arrows point to partial knockdown of CD44 in A. and CD147 in B., respectively. CD147 is variably glycosylated, giving diffuse bands at ~55kDa and 30kDa.

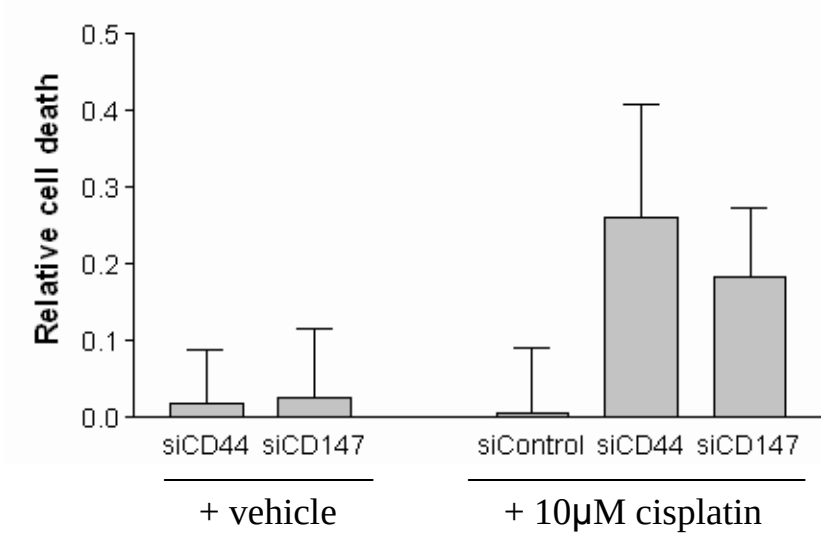

Figure 4. Chemo-sensitization *in vitro* of cisplatin-resistant ovarian carcinoma cells with siRNAs against CD44 and CD147 *in vitro*. A2780cp20 human ovarian carcinoma cells were incubated with pooled siRNAs against CD44 (siCD44) or CD147 (siCD147), or with control siRNA, as in Figure 3. The cells were then treated in the presence and absence of 10μM cisplatin for 72 h. Values represent fractional increase in cell death relative to untreated cells +/- S.D.

KEY RESEARCH ACCOMPLISHMENTS

1. Demonstration that small hyaluronan oligosaccharides sensitize cisplatin-resistant human ovarian carcinoma cells to cisplatin or doxorubicin treatment *in vivo.*
2. Demonstration that partial knockdown of CD44 or CD147 with siRNAs in cisplatin-resistant human ovarian carcinoma cells results in a partial increase in sensitivity to cisplatin treatment *in vitro*.

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have shown that small hyaluronan oligosaccharides sensitize cisplatin-resistant human ovarian carcinoma cells to cisplatin treatment *in vivo.* Recurrence of ovarian cancer in patients who have been given standard-of-care cisplatin treatment is a major cause of patient morbidity. Thus further investigation of the effects of small hyaluronan oligosaccharides, especially with respect to efficiency of delivery, is an important future objective.

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